## Online Methods

*Full methods are available in the supplementary information.*

**Primary cortical neurons and transfection.** Purified cortical neurons were isolated from E18 rat embryos and transfected at 5 DIV with Lipofectamine 2000. Experiments were carried out two days after transfection.

**Mice.** Experiments of EM microscopy and tomograpy, and the co-immune precipitation assays, were carried out on YAC18 (line 212) and YAC128 (line 53) mice generated to express full-length human huntingtin with 18 and 128 glutamines, respectively[13,14]. The animals were maintained on the FVB/N strain background. All experiments were approved by the Institutional Animal Care and Use Committee of University of Central Florida, College of Medicine.

**Imaging.** Fixed and live cell imaging were performed using an Axiovert Zeiss 100M inverted fluorescent microscope equipped with a Plan Apochromat 63×1.4 NA oil objective, a DG-4/Lambda 10-2 combo Xe-arc illumination unit (Sutter), and a Sensicam QE cooled CCD camera (PCO AG) controlled by MetaMorph 7.5 software (Molecular Devices). 3D images were acquired with the Multi Dimensional Acquisition module, 2×2 binning, 0.2 μm step size, and stacks of 15–20 z-planes. For live cell imaging, 1 μm step

size in 5 μm z-stack was acquired for each time point. Kymographs were generated with all planes using MetaMorph 7.5.

**Confocal microscopy.** For co-localization experiments fixed neurons were imaged using a NikonA1R VAAS microscope equipped with a spectral detector.

**Electron microscopy and tomography.** EM was carried out with a JEOL 1200FX electron microscope operated at 80 kV. Negatives were digitized at 1200 dpi with a Nikon CoolScan and micrographs were analyzed using Image J software. Mitochondrial length was measured as previously described[32]. Tomographic EM used a JEOL 4000 EX intermediate high-voltage electron microscope operated at 400 kV as previously described[15].

**Co-immune precipitation.** Samples were lysed in T-PER buffer (Thermo Scientific). Co-immune precipitations were performed using HTT-specific antibodies MAB2166 (clone 1HU-4C8, Millipore) or DRP1-specific polyclonal antibodies (Santa Cruz) followed by DRP1 (BD Bioscience) or HTT (clone 1HU-4C8, Millipore) Western blotting.

**DRP1 GTPase assay.** DRP1 GTPase activity was measured with the continuous method and in the presence of MOM liposomes and the data was analyzed as described[33].

**Statistical analysis.** Results are expressed as mean ± s.e.m. Data from two populations were compared using a Student's *t*-test, paired, two-sided. Data from multiple populations were analyzed with ANOVA post-hoc test.

**References for Online Methods**

32. Perkins, G.A.*, et al.* Electron tomographic analysis of cytoskeletal cross-bridges in the paranodal region of the node of Ranvier in peripheral nerves. *J Struct Biol* **161**, 469-480 (2008).
33. Ingerman, E. & Nunnari, J. A continuous, regenerative coupled GTPase assay for dynamin-related proteins. *Methods Enzymol* **404**, 611-619 (2005).

# Supplementary Information Titles

*Please list each supplementary item and its title or caption, in the order shown below.*

**Note that we do NOT copy edit or otherwise change supplementary information, and minor (nonfactual) errors in these documents cannot be corrected after publication.**
Please submit document(s) exactly as you want them to appear, with all text, images, legends and references in the desired order, and check carefully for errors.

| **Journal:** Nature Medicine |
|---|

| | |
|---|---|
| **Article Title:** | MUTANT HUNTINGTIN BINDS THE MITOCHONDRIAL FISSION GTPASE DRP1 AND INCREASES ITS ENZYMATIC ACTIVITY |
| **Corresponding Author:** | Ella Bossy-Wetzel |
| | |

| **Supplementary Item & Number (add rows as necessary)** | **Title or Caption** |
|---|---|
| Supplementary Methods | |
| Supplementary References | |
| Supplementary Figure 1 | The number of mitochondria decreases in neurons expressing mutant *HTT* |
| Supplementary Figure 2 | Mutant HTT increases the number of phagolysosomes. |
| Supplementary Figure 3 | Increased ratio of fission to fission plus fusion events in mutant *HTT* (Q46 and Q97) expressing neurons. |
| Supplementary Figure 4 | Mutant HTT exon1-Q97 co-localizes with DRP1 on mitochondria. |
| Supplementary Figure 5 | Specificity of the mutant HTT-DRP1 co-immune precipitations. |
| Supplementary Figure 6 | Mutant HTT does not form an increased complex with MFN2 in HD mice or HD individuals. |
| Supplementary Figure 7 | SDS-PAGE and Western blot analysis of human DRP1 protein expressed *in E. coli*. |

| | |
|---|---|
| Supplementary Figure 8 | DRP1 self-assembles in solution into ring- and spiral-like oligomers. |
| Supplementary Figure 9 | DRP1 knockdown by *DRP1* shRNA expression alters neuronal development of cortical neurons (7 DIV). |
| Supplementary Figure 10 | $DRP1^{K38A}$ rescue of mitochondrial transport defects in neurites of cortical neurons. |
| Supplementary Table 1 | Table of DRP1 and $DRP1^{K38A}$ GTPase enzymatic parameters including GTPase activities at 0.05 mM GTP, maximal rate of GTP hydrolysis ($V_{max}$), and apparent Michaelis-Menten constant ($K_m$). |
| Supplementary video 1 | Mitochondrial movement in a neuron expressing *HTT* exon1-Q17-GFP and DsRed2-Mito. Movie corresponds to the kymograph in **Fig 1f**, top panel and shows mitochondrial transport. The movie lasts 5 min and is played back accelerated (original: 5 s $frame^{-1}$, playback: 1/6 s $frame^{-1}$). |
| Supplementary video 2 | Mitochondrial movement in a neuron expressing HTT exon1-Q46-GFP and DsRed2-Mito. Movie corresponds to the kymograph in **Fig 1f**, center panel and shows a clear decrease in mitochondrial transport. The movie lasts 5 min and is played back accelerated (original: 5 s $frame^{-1}$, playback: 1/6 s $frame^{-1}$). |
| Supplementary video 3 | Mitochondrial movement in a neuron expressing HTT exon1-Q97-GFP and DsRed2-Mito. Movie corresponds to the kymograph in **Fig 1f**, bottom panel and shows more pronounced arrest in mitochondrial transport. The movie lasts 5 min and is played back accelerated (original: 5 s $frame^{-1}$, playback: 1/6 s $frame^{-1}$). |
| Supplementary video 4 | Electron tomography of a control mitochondrion in a medium spiny neuron. Movie showing the three-dimensional details of a mitochondrion in a medium spiny neuron reconstructed using electron tomography. These mitochondria are typically elongated along the direction of the axonal long axis. **Clip 1**: A rapid sequence through 190 slices (2.2 nm $slice^{-1}$) of the tomographic volume that shows nearly the entire mitochondrial volume. There are 84 cristae. **Clip2**: Rotations and zooms of the surface-rendered volume after segmentation of the inner and outer membranes. The blue outer membrane is translucent to visualize the cristae displayed in various colors. **Clip3:** Rotation of the cristae after removal of the outer membrane to better distinguish the variety of shapes and sizes. |

| | |
|---|---|
| Supplementary video 5 | Electron tomography of a fissioning YAC128 mitochondrion in a medium spiny neuron. Movie showing the three-dimensional details of a mitochondrion fissioning into three parts in a medium spiny neuron reconstructed using electron tomography. **Clip 1**: A rapid sequence through 210 slices (2.2 nm slice$^{-1}$) of the tomographic volume. There are 223 cristae, many of which are small. **Clip2**: Rotation showing the outer membrane and the widths of the two constriction sites. **Clip3:** Rotations showing the cristae in each of the three parts. **Clip4:** Rotations and zooms highlighting the cristae and the constriction sites. The blue outer membrane is translucent to visualize the cristae displayed in various colors. |